ly dealing with a series of coloured derivates—isomers and/or homologues—which are formed as the 11 double bonds react beside and one after another.

Trichloroacetic acid in concentrated aqueous solution dissolves carotene with a blue colour; in isobutylic alcohol, the colour is green. Both migrate towards the cathode.

Formic acid in glac. acetic acid or butyl alcohol yields blue, cationic carotene solutions. There appears to be at least one second coloured compound in solution, because a faint grey-blue zone remains stationary where the bulk of the blue colour has moved away.

Perchloric acid reacts in a remarkable way: if shaken with a benzene solution of carotene, the latter, not the former turns blue. So far as I know, this is the first epiphasic, lipophilic blue compound of a carotinoid with a strong acid. A second case is described in my subsequent note in this same issue. If the carotene solution is not extremely dilute, a solid with golden lustre separates from the blue benzene solution[3]. This blue benzene solution does not migrate in an electric field.

However, 5 vol.% $HClO_4$ in acetic acid or 20 vol.% in butyl alcohol yielded cationic blue solutions which changed their direction of migration twice in the course of some hours. Higher concentrations (of the 67% $HClO_4$ used) immediately gave anionic products.

Phosphoric acid slowly evolves a blue-green colour with carotene in butyl alcohol; this moves to the anode.

Sulfuric acid[1] also reacted quite differently according to its concentration and to the time of reaction. Thus 2·5 vol% in acetic acid gave a blue, cationic solution, whereas $H_2SO_4 \cdot H_2O$ with about 20% methanol yielded a blue colour that moved to the anode. Some combinations did not show migration at all, at least in the beginning. After a time violet and brown colours developed and the tendency became anionic.

To sum up: the ions formed with monobasic strong acids and β-carotene are primarily, that is in the beginning and at lower concentrations of acid, all cations. Polybasic acids yield sometimes anions, perhaps because they are attached to the molecule and make it anionic by their second dissociation. However, as seen with small amounts of sulfuric acid in acetic acid, the formation of cations may be the primary event in this case also. It is not clear from this point of view how anions could have been generated in some cases by the monovalent perchloric acid. It may be that anions and cations can be bound with alternating strength to the conjugated system.

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Department of Physical Chemistry, Technical University, Budapest, Hungary, February 6, 1956.

Zusammenfassung

Die Einwirkung von starken Säuren auf β-Carotin in geeigneten Lösungsmitteln führte zur Bildung von blauen Farbstoffen. Bei Anwendung von mono- und polybasischen Säuren wanderten diese neuen Stoffe meistens kathodisch, wenn die Säurekonzentration schwach war. Manchmal, vor allem bei stärkerer Konzentration oder nach längerem Stehen der Lösungen, wanderten sie anodisch.

[3] *Note added in the proof:* This solid is diamagnetic.

Action of Sulfuric Acid on β-Carotene

Concentrated solutions of β-carotene in benzene were shaken with ice-cold $H_2SO_4 \cdot H_2O$ under CO_2 and the blue solutions were decomposed with ice-water as quickly as possible. The yellow products were taken up in benzene, washed, dried and evaporated *in vacuo* to dryness. They were dissolved in benzene, and/or benzene petrolether mixtures, and chromatographed on a hydrated lime column or on a composite column made of a saccharose, a calcium carbonate and a hydrated lime section. We used petrolether (b.p. 60–70°C) or a mixture of the latter with ½ parts benzene as developers.

The sugar did not retain anything, the calcium carbonate layer retained an ochre-brown zone at its upper end, the rest developed several zones within the lime section. Development with petrolether split the mixture into more zones than was the case with benzene, especially at the bottom of the column. An upper red, thin zone was followed by a thin ochre one, then came a deep yellow-ochre, a thin red and two yellow zones one after another.

None of the zones showed the spectrum of β-carotene, despite the fact that the sulfuric acid reaction lasted only for 3 min. in one case. The boron trifluoride blue complex decomposed by ZECHMEISTER[1] did not behave in the same way; he could regenerate carotene if the reaction-time was only a few minutes. The lowest zone showed spectral maxima in CS_2 at 498, 467 and 440 mμ which correspond to a conjugated chain of 10 double bonds: 1 less than carotene. As no sulfur was detected in the eluted compound, a hydrolysis of the primary addition product must have occurred resulting in addition of one H_2O on a terminal double bond or in ω, ω'-position as in ZECHMEISTERS BF_3 experiments[2]. The other zones were examined in pentane solution and showed maxima at 426 (faint), 400, 375 and 345 mμ respectively. The zones did not seem to be sufficiently pure; their maxima were at the same wavelength irrespective of the fact that they belonged to distinct chromatographic zones of the column, but the relative heights varied from zone to zone. The maxima were shifted by 25–30 mμ towards longer wavelengths in CS_2. The eluted and concentrated carotinoids of each zone were brought once more into reaction with concentrated sulfuric acid. The top zone gave a violet colour, changing to pink. The red zone yielded a blue product, which was soluble in the benzene phase and left the underlying acid uncoloured. A similar case with β-carotene and perchloric acid was described in our preceding communication. These lipophilic blue acid-compounds of carotinoids seem to be absolutely unprecedented. The lower zones behaved normally in that they gave blue sulfuric acid solutions which changed to pink.

The fractions were neither sufficient nor pure enough to crystallize, but a generous gift of 20 g synthetic β-carotene from the Hoffmann La Roche Co., to whom I am very much obliged, will be of great help in this direction. Preliminary elementary analysis revealed that they contain oxygen.

I am about to isolate the "blue" compounds and separate them from each other if possible, to examine their decomposition products and general chemical, physical and magnetic properties.

[1] L. ZECHMEISTER, Exper. *10*, 1 (1954). – L. WALLCAVE, J. LEEMANN, and L. ZECHMEISTER, Proc. nat. Acad. Sci. *39*, 604 (1953).

[2] L. WALLCAVE and L. ZECHMEISTER, J. Amer. chem. Soc. *75*, 4495 (1953).

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Department of Physical Chemistry, Technical University, Budapest, Hungary, February 6, 1956.

Zusammenfassung

Die Einwirkung von Schwefelsäure auf eine benzolische β-Carotinlösung und die nachträgliche Behandlung der so gebildeten blauen Umwandlungsprodukte mit Wasser führte zu gelblichen Stoffen unbekannter Konstitution. Die chromatographische Analyse dieser Verbindungen zeigte die Abwesenheit von β-Carotin. Einige Eigenschaften der neuen Stoffe werden beschrieben.

Lipaemia Clearing Effect of Chlorpromazine

In the course of our investigations concerning the metabolic effects of chlorpromazine, it was observed that the plasma of the experimental animals became unexpectedly transparent after injection of the drug[1]. This effect resembled that exerted by heparin on lipaemic plasma first described by HAHN[2] in 1943. Our observation was supported by HOLLISTER and KANTER's[3] findings gained in two cases of essential hyperlipaemia: they found that chlorpromazine treatment decreased the turbidity of the sera of their patients and "produced a marked decrease in all fractions of blood fat, including a shift in the lipoprotein classes to a more nearly normal pattern", just like heparin treatment. On the basis of these findings, the question arose as to whether chlorpromazine exerts any effect on alimentary lipaemia?

Eleven mongrel dogs of both sexes were given 10 g/kg body weight of margarine orally after a 24 h fasting period. 3 h later, blood was taken from the femoral artery and chlorpromazine was given intravenously immediately afterwards in doses of 4–11 mg/kg body weight. In the course of the following 90 min, blood was withdrawn every 10 or 15 min from the femoral artery and mixed in a ratio of 1:9 with a 3·8% solution of sodium citrate. The blood samples were immediately centrifuged and the turbidity of the plasma recorded by means of a Pulfrich-photometer (Zeiss), with a S_{66} filter, against a water blank using ½ and 1 cm cells, respectively. All the figures obtained are given as optical density values of 1 cm cell-thickness.

After administration of chlorpromazine, the turbidity of the plasma decreased in every case. The decrease expressed in terms of optical density seems to a certain extent to be related to the chlorpromazine dose applied. The clearing effect was first observed 15–20 min after administration of the drug, the maximum was reached between 20 and 35 min. Afterwards a returbification occurred (Table I, Fig. 1).

In some of the cases, the changes in the lipoprotein pattern were recorded by means of paper-electrophoresis with the result that chlorpromazine caused a similar shift as heparin does (Fig. 2).

Table I

Dose of CPZ mg/kg	Optical density of the plasma before administration of CPZ	Maximal clearing effect %
4·0	0·615	26·81
5·0	0·690	38·20
5·0	1·980	38·80
5·0	0·420	91·00
6·0	1·206	45·30
8·0	2·240	10·26
8·0	0·850	53·00
9·0	0·772	58·30
10·0	1·920	37·20
11·0	1·270	98·74
12·5	0·927	73·80

Considering that these findings are very similar to those observed after small doses of heparin, it was assumed that chlorpromazine exerts its effect through mobilizing endogenous heparin. To support this assumption, the following experiment was carried out. 5 dogs were treated as described above, but 10 min before administering chlorpromazine, protamine-sulphate was given intravenously in a dose of 3 mg/kg body weight, all experimental conditions remaining unchanged. In each of the animals, protamine-sulphate inhibited the lipaemia clearing effect of chlorpromazine (Table II, Fig. 1).

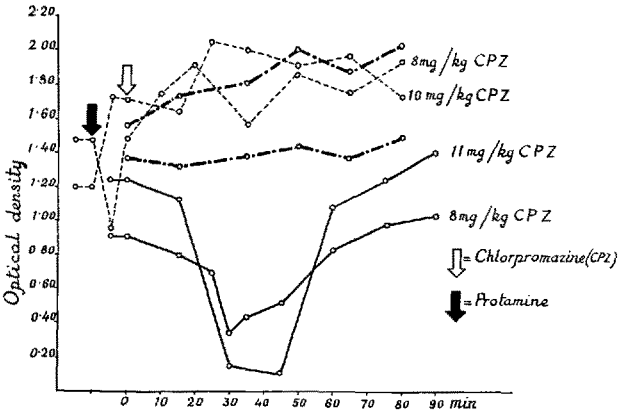

Fig. 1. —o——o——o = CPZ treatment only; o – – – – o = Protamine + CPZ treatment; o – · – · – · – o = no treatment.

HOLLISTER and KANTER suggest that the effect of chlorpromazine treatment in essential hyperlipaemia is due to the direct action of the drug on the liver; but they admit the possibility that the adrenolytic property of chlorpromazine may be responsible for the changes. Our experiments point to the fact that chlorpromazine exerts its lipaemia clearing effect via heparin, presumably by mobilizing endogenous heparin. Our hypothesis is supported by the fact that the clearing effect appears rapidly, bringing about a similar shift in the lipoprotein pattern as heparin, and that the effect is inhibited by protamine. PERLICK[4] reports that the complement-activity of the sera was decreased in patients during prolonged sleep-therapy produced by phenothiazine

[1] "Largactil", Specia, Paris.

[2] P. F. HAHN, Science *98*, 19 (1943).

[3] L. E. HOLLISTER and S. L. KANTER, Gastroenterology *29*, 1069 (1955).

[4] E. PERLICK, Langenbecks Arch. klin. Chir. *279*, 799 (1954).